In re: Boustany et al. Serial No.: 09/830,045 Filed: April 20, 2002

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In the Specification:

Please amend the specification as follows:

After the title, please insert the following paragraph:

--This application is a National Stage filing under 35 USC 371 of PCT/US99/24695, filed October 21, 1999, which claims priority to United States Provisional Application 60/105,262, filed October 22, 1998.--

Please replace the paragraph on page 17, lines 14–32 with the following paragraph:

-- RT-PCR: Messenger RNA was isolated from NT2 cells by the oligo-dT-cellulose method using the QuickPrep Micro mRNA Purification kit (Pharmacia Fine Chemicals, Piscataway, NJ). The mRNA was converted to cDNA by the reverse transcription (RT) reaction (Estus et al, 94). PCR reactions were set up with the first strand cDNA, 1U of Taq polymerase and 5 μ Ci of α^{32} P-dCTP in each reaction. The reactions were performed in Tag buffer containing 1.5 mM MgCl₂, 2.5 mM dCTP and 5 mM each of dATP, dGTP and dTTP. The primers used for amplification of CLN3 were 5' primer: 5'-GGTGGACAGTATTCAAGGG-3' (958–976, SEQ ID NO: 1) and 3' primer: 5'-CTTGGCAGAAGACGAAC-3'(1229-1246, SEQ ID NO: 2). Cyclophilin was used as the internal control and primers used for cyclophilin amplification were the 5' primer: 5'-AAATGCTGGACCCAACAC-3' (317-334, SEQ ID NO: 3) and 3' primer: 5'-AAACACCACATGCTTGCC-3' (384-401, SEQ ID NO: 4). The reaction conditions used were 1 minute at 94°C, 1 minute at 50°C and 2 minutes at 72°C for 20 cycles. The PCR amplified products were analyzed on an 8% non-denaturing polyacrylamide gel that was dried and visualized by autoradiography. The amplified signal was quantitated using a PhosphorImager (Molecular Dynamics Inc., Sunnyvale, CA). The results are expressed as the ratio of CLN3 signal to that of the internal control cyclophilin. This provides a reproducible and comparative, semi-quantitative measure of CLN3 expression. --

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Please replace the paragraph on page 18, lines 1–20 with the following paragraph:

-- Western Blotting for CLN3 and PARP detection: The CLN3 antibody used in this study is a polyclonal antibody raised against the peptide sequence AAHDILSHKRTSGNQSHVDP (SEQ ID NO: 5) corresponding to amino acids 58-77 of the CLN3 protein (Research Genetics, Huntsville, AL). Total cellular extracts for CLN3 detection were prepared from NT2 cells transfected with CLN3 or the appropriate vector control. Cells were washed with cold PBS and lysed in buffer (250 mM NaCl, 0.1% NP40, 50 mM Hepes, pH 7.0, 5 mM EDTA, 1 mM DTT, 1 mM PMSF) on ice for 10 min. The lysate was collected and clarified by centrifugation at 12,000xg for 10 min at 4°C. The supernatant was quantitated for total protein by the BioRad protein assay method. Equal amounts of total protein from each sample were electrophoresed on a 9% SDS-polyacrylamide gel in buffer containing 0.092 M glycine, 0.125 M Tris-OH and 2% SDS. The gel was transferred onto nitrocellulose membrane by semidry electroblotting and blocked by incubation in solution containing 3% BSA in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20) buffer for one hour at 25°C followed by incubation with the CLN3 antibody (IgG fraction) diluted in TBST, for 15 hours at 25°C. After extensive washes with TBST buffer, the membrane was incubated in a 1:5000 dilution of goat-anti-rabbit IgG conjugated with horseradish peroxidase for 30 minutes at 25°C. The blot was washed and developed using the chemiluminescence detection system (Amersham, Arlington Heights, IL). --

Please replace the paragraph on page 27, lines 16–21 with the following paragraph:

- -- Primers used for PCR amplification of human CLN3 were:
- 5' primer: 5'-GGTGGACAGTATTCAGGG-3' (958–976, SEQ ID NO: 1)
- 3' primer: 5'-CTTGGCAGAAAGACGAAC-3' (1229–1246, SEQ ID NO: 2)

Primers used for amplification of human cyclophilin amplification:

- 5' primer: 5'-AAATGCTGGACCCAACAC-3' (317–334, SEQ ID NO: 3)
- 3' primer: 5'-AAACACCACATGCTTGCC-3' (384-401, SEQ ID NO: 4)